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Short communication

## Epidemiology of canine distemper and canine parvovirus in domestic dogs in urban and rural areas of the Araucanía region in Chile

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### ABSTRACT

To assess whether the seroprevalence of canine distemper virus (CDV) and canine parvovirus (CPV) in domestic dogs is higher in urban versus rural areas of the Araucanía region in Chile and risk factors for exposure, a serosurvey and questionnaire survey at three, urban-rural paired sites was conducted from 2009 to 2012. Overall, 1161 households were interviewed of which 71% were located in urban areas. A total of 501 blood samples were analysed. The overall CDV and CPV seroprevalences were 61% (CI 90%: 58–70%) and 47% (CI 90%: 40–49%), and 89% (CI 90%: 85–92%) and 72% (CI 90%: 68–76%) in urban and rural areas, respectively. The higher seroprevalence in domestic dogs in urban areas suggests that urban domestic dogs might be a maintenance host for both CDV and CPV in this region. Due to the presence of endangered wild canids populations in areas close to these domestic populations, surveillance and control of these pathogens in urban dog populations is needed a priority.

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## 1. Introduction

Domestic dogs are particularly abundant in urban areas of some developing countries where they can act as efficient reservoirs for pathogens because they usually live in large populations, are not vaccinated and are regularly allowed to roam freely, facilitating contact between infected and susceptible hosts (Amaral et al., 2014). In rural areas where domestic dog densities and population size are often low, highly virulent pathogens such as rabies and canine distemper virus (CDV) cannot be maintained and it is expected that these infections fade out without the introduction of new infections from neighbouring areas (Funk et al., 2001; Lembo et al., 2008). Despite the risk of spillover of CDV and canine parvovirus (CPV) from urban domestic dogs populations to wild canids – including indirectly via contaminated environments (Gordon and Angrick, 1986) – few studies have been conducted to investigate this in the field (e.g. Acosta-Jamett et al., 2011; Frölich et al., 2005).

The Araucanía region of Chile is inhabited by three wild canids: the culpeo (*Lycalopex culpaeus*), the chilla (*Lycalopex griseus*) and

one of the most endangered canid species on the world, Darwin's fox (*Pseudalopex fulvipes*), and inhabits the Nahuelbuta National Park (NNP) (37°47'S, 72°59'W; Jiménez et al., 2008). Recent studies have reported its presence in the locality of Lastarria (Gorbea district, 39°11 S, 72°6'W; D'elía et al., 2013) and other areas south to the NNP (Farias et al., 2014). Whether domestic dog populations from urban areas in the Araucanía region are the source of CDV or CPV infection in the region is unknown. In this study our goal was to determine the seroprevalence and risk factors for CDV and CPV infection in domestic dogs in urban and rural areas of the Araucanía region and to assess the risk to wild canids by targeted sampling in areas where spillover of infection might occur within this fragmented landscape.

## 2. Material and methods

### 2.1. Study area

The study was conducted in the Araucanía region in South Central Chile. To compare CDV and CPV seroprevalence in dogs at urban and rural sites the study included three urban areas – Angol (~50,000 inhabitants), Curacautín (~16,000 inhabitants) and Gorbea (~14,000 inhabitants) – and rural areas located close to these cities and three places where wild canids were known to

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occur. These sites included Nahuelbuta National Park (37°45'S; 73°00'W), Conguillío National Park, 15 km south-east of Curacautín (38°42'S; 71°37'W); and Arauco forestry company lands, located close to Lastarria locality, 20 km south-west of Gorbea (39°11'S; 72°46'W) (Fig. 1).

## 2.2. Sampling design

Previous studies carried out in urban areas of Chile have reported CDV and CPV seroprevalences of 75% (Acosta-Jamett et al., 2011) and 82% (Acosta-Jamett, 2009), respectively. To estimate the total population of dogs a human:dog ratio of 5.5:1 was assumed. Target sample size in cities was 50 dogs (assumed conservative seroprevalence 75%, desired absolute precision 10%, 90% confidence; EpiInfo 7). A sampling design similar to that of Acosta-Jamett et al. (2010) was used, randomly sampling households within cities and visiting all households within each rural area and sampling at least one dog in each household (Fig. 1).

## 2.3. Questionnaire survey and dog sampling

A questionnaire was conducted between 2009 and 2012 and was developed following similar studies (Acosta-Jamett et al., 2010; Kitala et al., 2001). All selected households were visited and re-visits were done if no household member was available at the first visit. Only adult members of the household were interviewed. The questionnaires were asked in Spanish by a team of veterinarians and veterinary undergraduate students, who were trained and supervised by the lead author.

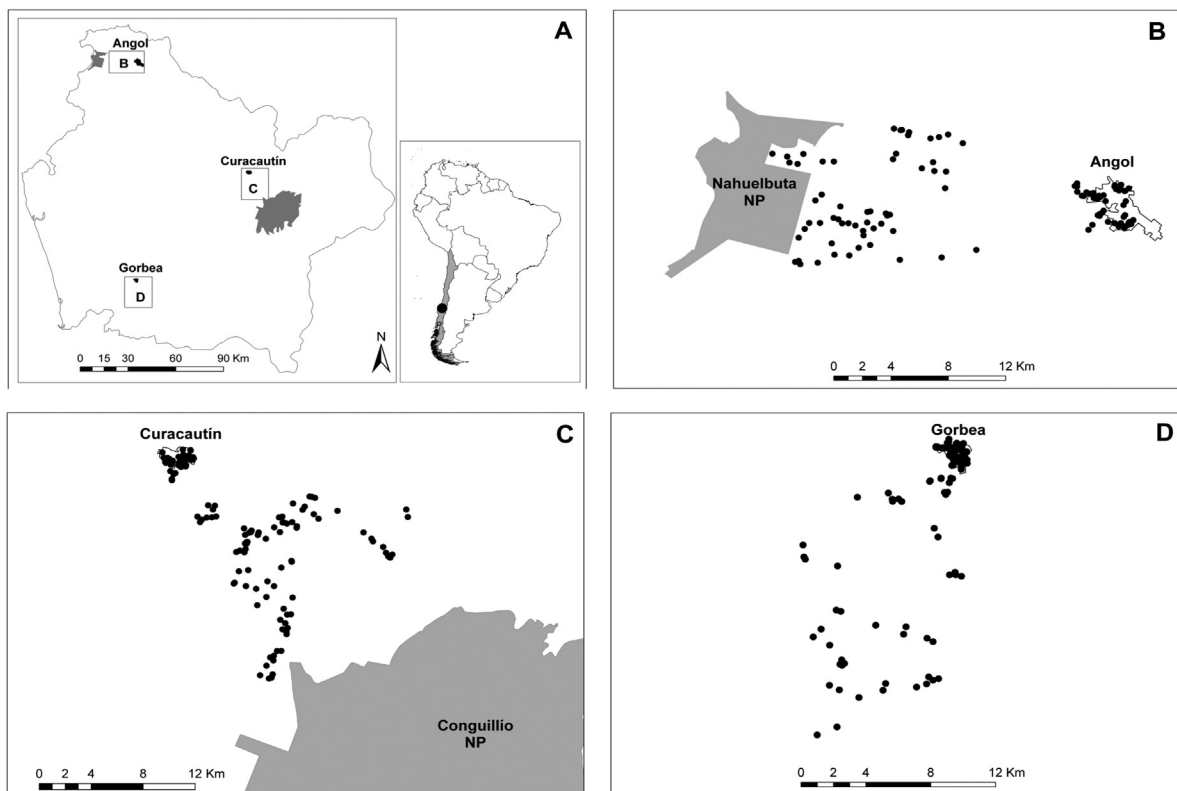
Questions within the questionnaire can be divided into two levels: (1) household level, and (2) animal level. The data collected at the household level was 'number of people per

household', 'number of dogs per household', 'methods of feeding' (commercial/household food), 'waste disposal methods' (burning, burial, municipality disposal), 'education of owners' (primary, secondary, superior) and 'household condition' (owners, leasing, family home). At the animal level questions included 'age' (<12, 12–24, >24 month), 'sex' (male/female), 'purebred' (yes/no), 'origin' (gift, born at home, found), 'function' (pet, guarding, herding), 'allowed to roam freely' (always, sometimes, never), 'seen by veterinarian' (yes/no), 'anthelmintic treatment in last three months' (yes/no) and 'spayed/neutered' (yes/no). Questions regarding the vaccination status of each animal were made in order to sample only unvaccinated animals. All data was transferred into a database using unique identifier numbers at the household and individual level. Data was kept as confidential.

The coordinates of each household were recorded with a GPS (Etrex, Garmin®) and then transferred to a GIS system (ArcGIS 10.1). Three additional spatial risk factors were included in the analyses: 'distance to city', 'distance to nearest household', and 'distance to road' by using a proximity function which measures the Euclidian distance between the household of each sampled dog and the center of the nearest city, household or road, respectively.

## 2.4. Demography of domestic dogs

With the information obtained during the questionnaire survey we calculated the percentage of dog-owning households (DOHH). We also estimated the average number of dogs per household by summing the dogs reported in each study site and dividing by the interviewed households. In addition, the human:dog ratio was calculated by summing the total number of people reported in each urban or rural site and dividing by the total number of dogs reported for each site (Acosta-Jamett et al., 2010). Following



**Fig. 1.** (A) Study area in the Araucanía region in south-central Chile where a questionnaire survey of domestic dog-owners at three sites was conducted (in rectangle). In black are urban areas included in the study and in grey are protected areas. (B–D) Selected sites where sampling was carried out. Black dots represent households where blood samples were obtained from domestic dogs. In grey are shown Nahuelbuta National Park (B) and Conguillío National Park (C).

Acosta-Jamett et al. (2010), we calculated the density of dogs in each site by first estimating the domestic dog population by dividing the human population size reported in the human census of 2002 (INE, 2005) by the human:dog ratio obtained in our study. Then the dog population size was divided by the area of each site i.e. a polygon created by the lines connecting the most external interviewed households at that site (ArcGIS 10.0).

### 2.5. Serum antibody testing

At the interviewed households dogs were manually restrained to obtain blood samples. Blood collected from the cephalic vein was deposited in plain 5 ml vacuutainers and centrifuged the same day with a Mobicentrifuge. The serum was stored at  $-18^{\circ}\text{C}$  in electrical freezers in the field until analysis. Serum samples were analyzed using the ImmunoComb® dot-ELISA kit (Biogal-Galed Laboratories, Kibbutz Galed, Israel), a commercial kit with 95.5% specificity and 93.1% sensitivity (Waner et al., 2003).

### 2.6. Data analysis

Chi-square and Fisher exact tests were used to compare dog-ownership between urban and rural areas and CDV and CPV seroprevalence in dogs between urban and rural sites. The associations between potential risk factors and seropositivity (positive/negative based on the recommended cut-offs) to CDV and CPV were estimated using a multivariate logistical regression analysis (R 2.12 statistical software). Additionally, Moran's I test was used to assess whether spatial clustering existed in each of the three study sites for CDV and CPV exposure (ArcGIS 10.0 Spatial Statistics). Finally, clustering at each rural and urban site for CDV and CPV separately was further investigated by Cuzick and Edwards' test for inhomogenous populations. In this analysis, binary data (seropositive, negative) and up to the 6th nearest neighbour was considered. The significance of spatial clustering was assessed by calculating a z-statistic (Ward and Carpenter, 2000).

## 3. Results

### 3.1. Demography and vaccination of domestic dogs

Overall, 1161 households were interviewed of which 71% were located in urban areas. The proportion of dog-owning households was lower in urban (50%, range 44–57%) than in rural (98%, range 97–100%) sites. The human:dog ratio was ~4 times lower in rural than in urban areas and higher numbers of dogs per household were found in rural than in urban sites (Table 1). Comparing the frequency of dog-owning households, statistical differences were found in urban areas ( $p < 0.05$ ), which ranged between 44 and 57%, but no differences were detected in rural areas ( $p > 0.05$ ), where proportions reached nearly 100% in the three sites. Overall,

vaccination coverage was reported to be 42% and 8% in urban and rural areas, respectively (Table 1).

### 3.2. Seroprevalence of CDV and CPV

A total of 501 blood samples of unvaccinated dogs were collected in the interviewed households. The overall CDV seroprevalence was 52% (257/500) and ranged from 36% to 80% depending on the site. Higher CDV seroprevalence was found in domestic dogs from urban than rural areas ( $p < 0.01$ ). The overall CPV seroprevalence was 78% (391/500) and ranged from 65% to 92%. Similarly, higher overall CPV seroprevalence in dogs was found in urban than rural areas ( $p < 0.001$ ).

### 3.3. Risk factors in domestic dogs

CDV seropositive dogs were about 3-times more likely in urban than rural sites and this increased with age and was higher with increasing numbers of dogs per household (Table 2). CPV seropositivity was higher in Angol than in Gorbea but not different between Angol and Curacautín. Similarly to CDV, there was a higher probability of CPV seropositive dogs being in urban than

**Table 2**

Multivariate logistic regression model of the factors explaining canine distemper virus and canine parvovirus seropositivity in domestic dogs ( $n = 501$ ) across the study sites in the Araucanía region, Chile.

Risk factor	Coefficient	OR	p
<b>Canine distemper virus</b>			
Site			
Rural	–	1	
Urban	1.07	2.9	<0.001
Age			
<12 Months	–	1	
12–24 Months	1.66	5.2	<0.001
>24 months	2.72	15.2	<0.001
Number of dogs per household	0.23	1.3	<0.05
<b>Canine parvovirus</b>			
Area			
Angol	–	1	
Curacautín	–0.09	0.9	0.79
Gorbea	–0.91	0.4	0.02
Site			
Rural	–	1	
Urban	1.36	3.9	<0.001
Age			
<12 months	–	1	
12–24 Months	1.71	5.5	<0.001
>24 Months	1.82	6.2	<0.001
Function			
Hunting/herding	–	1	
Pet/guarding	–0.90	0.4	0.04

**Table 1**

Patterns of dog ownership observed from a questionnaire survey in the Araucanía region, Chile 2009–2012.

Variables	Angol		Curacautín		Gorbea		Total	
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural
Interviewed households	309	135	215	145	300	57	824	337
DOHH <sup>a</sup> (%)	57	98	44	97	50	100	50	98
Vaccination (%)	45	2	42	16	38	3	42	8
Dogs per household	0.9	2.6	0.7	2.6	0.9	3.0	0.8	2.7
Human:dog ratio	4.2	1.3	4.3	1.2	3.9	1.0	4.1	1.2
Density (dogs/km <sup>2</sup> )	1240	2.5	785	3.7	1631	0.84	1634 <sup>b</sup>	2.4 <sup>b</sup>

<sup>a</sup> DOHH: dog-owning households.

<sup>b</sup> Average density.

rural sites. Also, age was a factor predicting CPV exposure. Finally, dogs performing hunting or herding functions were more likely to be CPV seropositive than pet or guarding dogs (Table 2).

When assessing whether clustering was present in domestic dogs exposed to CDV or CPV in any of the three areas, there was no significant clustering except for CPV at the Angol site ( $z=2.23$ ,  $p=0.03$ ), which indicated clustering in the center of the urban area. For the Cuzick and Edwards' test, the only clustering found was for seropositivity to CPV at the Curacautín site. At this site, in urban areas CPV seropositive dogs were more likely to have another CPV seropositive dog as either the nearest ( $z=2.17$ ,  $p=0.03$ ) or second nearest neighbour ( $z=2.50$ ,  $p=0.01$ ). In rural areas, CPV seropositive dogs were more likely to have another CPV seropositive dog as their second nearest neighbour ( $z=2.08$ ,  $p=0.03$ ). These results indicate a wide presence of domestic dogs exposed to CDV and CPV in both urban and rural areas across the region, with little evidence of spatial clustering.

#### 4. Discussion

Domestic dogs from urban areas had a higher risk of being CDV and CPV seropositive than rural dogs, suggesting increased force of infection in urban dogs. This is probably due to differences in demography: urban areas with a higher density of domestic dogs and increased contact rates. The higher seroprevalence in adult dogs could be caused by (a) a constant force of infection in an endemic area, (b) differential rates of exposure in a population experiencing sporadic outbreaks, (c) an increase in disease exposure with age, or (d) a recent epidemic. Similar age-seroprevalence patterns have been reported for CDV (e.g. Acosta-Jamett et al., 2011) and CPV (e.g. Acosta-Jamett, 2009) worldwide. Additionally, the reduction in seroprevalence from urban to rural dogs suggests a likely directional reduction in exposure to CDV and CPV following a city–village model (Grenfell and Bolker, 1998). This is consistent with what was found by Acosta-Jamett et al. (2011) for CDV in northern Chile. This could indicate that in these urban areas dogs are the reservoir of pathogens for rural dogs, since population sizes are well above the critical community size for disease transmission compared to domestic dog populations in rural sites. The CDV and CPV seroprevalences in domestic dogs in urban and rural areas estimated in this study are similar to what has been reported previously in northern and southern Chile (Acosta-Jamett, 2009; Acosta-Jamett, 2009, 2011; Acosta-Jamett et al., 2014; Sepulveda et al., 2014), and as expected we found higher seroprevalences of both pathogens in urban than rural areas (Acosta-Jamett, 2009; Acosta-Jamett, 2009).

Although CPV can be maintained in the environment for months (McCaw and Hoskins, 2006), a recent study has shown that CPV can be transmitted not only by direct contact but also through vectors such as flies (Bagshaw et al., 2014). A high amount of waste in urban areas could lead to higher concentration of flies at these sites than in rural areas, which could be an additional factor explaining the differences found in this study. Although we were not able to find any spatial factor related to CDV or CPV seropositivity, this can be explained by the fact that dogs that are commonly left abandoned in rural areas originate from cities (Acosta-Jamett et al., 2011), thus producing a gradient of exposure of dogs to CDV or CPV from urban to rural sites. Domestic dogs from high-density populations are suggested to be the source of infection to wild carnivores. Some studies have found that epidemics that later affected wild canids started in domestic dogs in urban settings, for example a CDV outbreak in Kenya (Alexander and Appel, 1994) and in Namibia (Gowtage-Sequeira et al., 2009) that affected jackals.

Study limitations include the use of serological tests that could produce false positive results due to cross-reaction with other agents, detecting only exposure and not actual infection, and which could lead to misinterpretations. According to the spatial analyses domestic dogs exposed to both CDV and CPV are present even in close proximity to protected areas and sites where wild canids are reported to occur (D'elía et al., 2013; Farias et al., 2014; Jiménez et al., 2008). Based on data obtained during our cross-sectional serosurvey we cannot determine if CDV and CPV spill over from the domestic dog population to wild carnivores in the Araucanía region. However, the higher seroprevalence in urban compared to rural domestic dogs suggest a possible directionality of infection from urban to rural areas.

Urban areas have abundant domestic dog populations in many developing countries and in Latin America we can find some of the largest urban populations worldwide; domestic dog population density in Chilean cities is amongst some of the highest in Latin America (Acosta-Jamett et al., 2010; Gompper, 2014). Further plans for disease surveillance should be implemented to detect outbreaks of CDV and CPV in domestic dog populations near endangered species and plans for actively controlling these diseases through population management and vaccination of free-roaming dog populations and thus increasing herd immunity to reduce the  $R_0$  is recommended.

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